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TIME-TEMPERATURE EFFECTS ON SALMONELLAE
AND STAPHYLOCOCCI IN FOODS. I. BEHAVIOR
IN BROTH CULTURES AND REFRIGERATED FOODS

Robert Angelotti, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

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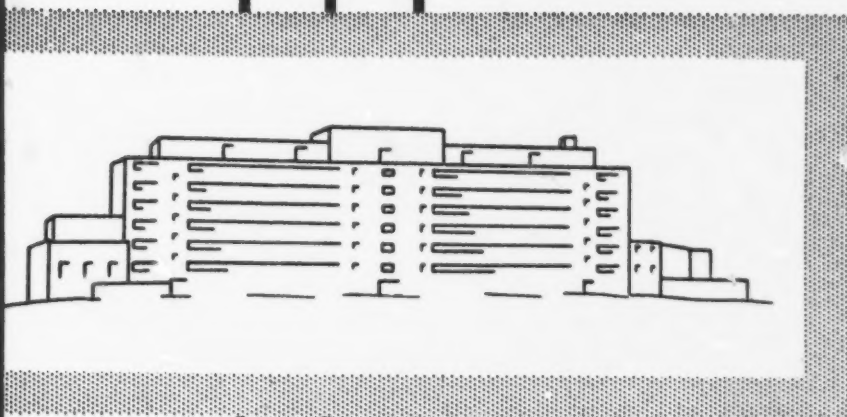
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I. Behavior in Broth Cultures and
Refrigerated Foods.

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TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

I. Behavior in Broth Cultures and Refrigerated Foods

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Milk and Food Research Program

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Robert A. Taft Sanitary Engineering Center
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TIME-TEMPERATURE EFFECTS ON SALMONELLAE AND STAPHYLOCOCCI IN FOODS

ABSTRACT

Staphylococci and salmonellae are the principal causes of illness currently encountered in perishable foods. Time-temperature data comparable to those available for the pasteurization of milk or for canning, which define conditions of refrigeration, cooking, or warm holding of perishable foods, have not been established for these organisms. These studies were undertaken to make available an organized body of technical data on the critical time-temperature values for salmonellae and staphylococci under these conditions.

Initial experiments were performed to determine the response of several enterotoxigenic staphylococci and food-poisoning salmonellae in brain heart infusion broth to temperatures ranging from 41°F to 158°F. These experiments served to orient the investigators as to the temperature limits at which growth, survival, or death of the test strains occurred; to aid in selecting a few strains of both types of organisms with characteristic temperature responses; and to define the temperatures and sampling intervals suitable for subsequent studies in foods.

The following species of *Salmonella* were selected for use in the broth studies: *S. manhattan*, *S. enteritidis*, *S. typhimurium*, *S. senftenberg*, *S. cubana*, *S. montevideo*, and heat-resistant strains of *S. typhimurium* 6087 and *S. senftenberg* 775W. Eight enterotoxigenic strains of *Staphylococcus aureus* also were employed. They were designated as Ms 149, 21 B, 196 E, MF 159, 224, 2Y, D 13, and 161 C. One ml. aliquots of suspension of each of the test organisms prepared in phosphate buffered dilution water were inoculated into duplicate tubes, each containing 10 ml. of sterile Bacto brain heart infusion broth and pre-adjusted to the desired test temperature. The inoculated tubes contained approximately 10,000,000 cells per ml. and were incubated in water baths adjusted to the test temperatures. At various intervals, depending upon the temperature, turbidity measurements and plate counts were made of the salmonellae and staphylococcal broth cultures.

The results indicated that neither group of organisms multiplied at 41°F, and that good growth of all the salmonellae occurred at 50°F, but only fair growth was observed on the part of six of eight staphylococcal strains at this temperature. Good growth of both groups was also noted at 59°F and 68°F.

With one exception, excellent growth of all the test cultures was observed at 113°F, and the maximum cellular concentrations were attained between four and eight hours' incubation. Staph. aureus MF 159 grew very slowly at this temperature.

No growth of any of the test cultures occurred at or above 122°F; however, the staphylococci survived at higher temperatures and for longer time intervals than the salmonellae.

Based on their reaction to temperatures in broth, the following cultures were selected as showing varying heat resistance and ability to survive cold temperatures: S. senftenberg 775W, S. enteritidis, and S. manhattan high, medium and low heat resistance respectively. Minor differences in heat resistance were observed among the staphylococci, with Staph. aureus Ms 149 displaying a slightly greater heat resistance than strains 196E and MF 159.

Additional studies were performed in order to supply information on the growth response of salmonellae and staphylococci at 40°F to 50°F in foods representative of the types associated with food-poisoning outbreaks.

Based on the results obtained from the broth studies, a pooled suspension of S. senftenberg 775W, S. enteritidis and S. manhattan and a separate pooled suspension of Staph. aureus Ms 149, Staph. aureus 159, and Staph. aureus 196E were used to inoculate sterile, 50 g. aliquots of ham salad, chicken a la king, and custard contained in 2 oz. screw cap jars which were previously adjusted to the desired test temperature. Mixed cultures were used as inocula because it was felt that suspensions containing organisms of varying heat resistance would represent more closely the response to temperature of the two groups of organisms than that possible by the use of pure cultures. In addition, the use of multiple strains minimized the importance to the final result of a given food affording a single strain, an unknown substrate specificity which might affect its response to temperature. The inoculated jars of food were incubated for 5 days at 2°F intervals from 40°F to 50°F and at 95°F. Plate counts were made of the inoculated foods at 24-hour intervals.

The results revealed that in custard the staphylococci grew at temperatures of 44°F and above. The salmonellae underwent a gradual decrease in numbers at all temperatures from 40°F through 50°F.

In chicken a la king, growth of both salmonellae and staphylococci occurred at temperatures of 44°F and above.

In ham salad, no growth of either group of organisms occurred at 40°F through 50°F.

Good growth of both groups of organisms was observed in all three types of food incubated at 95°F.

These data indicated that if perishable foods are to be stored for extended periods, internal temperature of the food should be maintained at or below 42°F in order to prevent the growth of salmonellae and staphylococci.

INTRODUCTION

Most foodstuffs are perishable and therefore subject to spoilage and deterioration. Unless perishable foods are refrigerated properly or otherwise preserved from the time of harvest to their final preparation for table service, they may become vehicles for the growth of food-poisoning and other infectious microorganisms. Individuals concerned with the handling of perishable foods should be constantly alert for the dangers involved and take the appropriate measures necessary to maintain food in a wholesome condition.

Epidemiological examination of foodborne infections and intoxications usually shows that the foods in question are heavily contaminated and have been maintained at temperatures favorable to multiplication for periods which would allow extensive growth of the microorganisms involved. Thus, time and temperature are found to be interdependent factors requiring rigid control in order to prevent the transmission of disease by food. Infectious and toxigenic microorganisms, when present, may survive inadequate heat treatments and multiply rapidly if the foods are held at temperatures suitable for growth.

The popularity of precooked, ready-to-serve foods, the increased use of automatic vending machines for dispensing perishable meals and the acceptance of convenience foods, in general, have complicated the time-temperature control problems facing public health workers who are engaged in the prevention of foodborne diseases.

There is need for the development of information concerning the role of various ecological factors important in the transmission of foodborne disease. Temperature control has been long recognized as an environmental factor important in food sanitation and has been widely applied on the basis of practical experience. There is not, however, an organized body of technical data on the critical time-temperature relationships applicable to the safe handling of perishable foods during processing, storage, preparation, and serving. For these reasons, studies have been undertaken to collect the basic data needed for the development of sound recommendations that can be used in ordinances, codes, and manuals of operation concerning safe practices of food handling.

STATEMENT OF THE PROBLEM

Because of research performed in the past, the effect of time-temperature relationships on the life processes of bacteria are well understood. However, there is a current need for specific information on the application of these fundamentals to modern food-handling activities. Unless food-handling practices are based on laboratory studies of the effect of time and temperature on the growth, survival, or death of bacteria in foods, rather than upon empirical judgment, foods will play a continuing role in the spread of disease.

Staphylococci and salmonellae are the principal causes of illness currently encountered in perishable foods. Time-temperature data for these organisms comparable to those available for the pasteurization of milk or for canning, which define conditions of refrigeration, cooking or warm holding of perishable foods, have not been established. This study was undertaken to make available critical time-temperature values for salmonellae and staphylococci under these conditions.

HISTORICAL REVIEW

As man developed a community existence, he sought ways of building up food reserves. Many of the preservation methods in use today have origins lost in antiquity. Though drying, salting, and smoking were known to the ancient Egyptians and Romans (Van Loesecke 1949), it was not until the 19th and 20th centuries that scientists discovered the mechanism by which these methods preserved foods and showed the relationships between food-spoilage, foodborne disease, and microbiological activities. Through their efforts food sanitation was developed and the concept of heating or cooling foods to destroy or retard microbiological activity was placed on a scientific basis. Although it was late in man's history that the application of heat or cold for preserving foods was understood, the effect of temperature on biological activity was recognized much earlier.

BOILING

Boiling must have been recognized as an early means of destroying microbial life, for Joblot employed it to sterilize hay infusions in 1710 during his experiments on the spontaneous generation of life (Frobisher 1957). Needham in 1745 and Spallanzani in 1769, during their controversy concerning spontaneous generation, boiled flasks of putrescible fluids to rid them of pre-existent life (Topley and Wilson 1953). However, it was not until the Napoleonic Wars and the impetus of a prize of 12,000 francs offered by the French government for a method of preserving food to feed the Emperor's army, that Nicolas Appert, in 1810, announced his discovery of heating foods in hermetically sealed cans to preserve them. However, Appert did not recognize the biological nature of decay. Some years after, Pasteur began his studies on fermentations and showed that the fermentation of organic fluids was always associated with the presence of living cells. Between 1860 and 1864 (Topley and Wilson 1955), he studied the "diseases" of wine and noted that souring and abnormal fermentations could be prevented by heating the wine for a few minutes to 122°F to 140°F. After studying the "diseases" of beers, he recommended a heating process for this beverage as well, and the new term "Pasteurization" soon became current in the language. With the establishment of the "germ theory" and the knowledge that heat was capable of killing microorganisms, the influence of temperature on the life processes and death of bacteria came under intensive study.

THERMAL DEATH POINTS

The complete destruction of bacteria by heat was first studied by Koch, et al. (1881). In a series of carefully planned experiments, he laid down the fundamental principles of heat sterilization in use today. In his early studies on anthrax he found that it required three hours' exposure in air heated to 284°F to destroy the spores, but that they were killed in just a few minutes by boiling. He also showed that steam under pressure produced higher temperatures and effected sterilization more rapidly than boiling or flowing steam.

From the time of Koch, the thermal death points of pathogenic bacteria have been studied extensively because of their practical importance in hygiene and medicine. Through the efforts of early workers there developed a body of information which revealed that the thermal death points of bacteria were affected by such things as the medium in which the organisms were grown, the menstruum in which the organisms were suspended, cellular concentration, incubation temperature, pH, age of culture, test method, whether heating was performed in sealed ampules or open tubes, and that all of these factors must be considered in accepting a given temperature as a thermal death point of the organism in question (Gotschlich 1917).

The mode of action of heat on bacteria appears to be one of protein coagulation. Chick (1908) showed that heat coagulation of proteins is an orderly process and consists of two stages, denaturation and agglutination. The rate of coagulation is logarithmic and is proportional at any moment to the concentration of uncoagulated protein. The higher the temperature to which bacteria are subjected the more rapid is the coagulation of their protein. Chick (1910) also showed that the death of bacteria by heat did not occur at a definite point on the temperature scale but over a range of temperatures; and that death of bacteria is a function of time.

PASTEURIZATION OF MILK

At the turn of the century the pasteurization of milk was recognized as a means of destroying the various pathogenic bacteria often associated with it (Rosenau 1909). Mycobacterium tuberculosis was determined to be the most heat resistant of the many pathogens found in milk; consequently, the recommended time and temperatures for pasteurization were based on the heat resistance of this organism. Early conclusions on the temperatures required to kill M. tuberculosis were quite variable. Theobald Smith (1899) explained these differences on the basis that the organisms contained in the pellicle, formed on the surface of milk due to evaporation during heating, were protected from the lethal temperature. He found that 140°F killed M. tuberculosis in 15 minutes if no pellicle was allowed to form, but with a pellicle it might survive 140°F for 60 minutes. Rosenau (1908) in a study of the thermal death points of bacteria in milk, reviewed the results of many investigations on the thermal death point of the tubercle bacillus in milk. These results ranged from the extremes of

momentary exposure at 212°F to 10 minutes' exposure at 140°F to cause death of M.tuberculosis. Until 1927 confusion existed over the exposure time and temperature necessary to kill this organism in milk. The work of North and Park (1927) contributed much to resolve the situation. As a result of their carefully conducted studies, it was concluded that a minimum temperature of 142°F for a minimum time of 30 minutes destroyed M.tuberculosis in milk and afforded an ample margin of safety.

The United States Public Health Service presently defines pasteurization in the Milk Ordinance and Code as: "The terms 'pasteurization,' 'pasteurized,' and similar terms shall be taken to refer to the process of heating every particle of milk or milk products to at least 143°F, and holding it at such temperature continuously for at least 30 minutes, or at least 161°F and holding at such temperature continuously for at least 15 seconds, in approved and properly operated equipment: Provided, that nothing contained in this definition shall be construed as barring any other process which has been demonstrated to be equally efficient and which is approved by the State health authority."

Recent studies on pasteurization conducted by Enright, Sadler, and Thomas (1957) revealed that Coxiella burnetii, the causative agent of Q-fever, is more heat resistant than other pathogens likely to be in milk. On the basis of their results, the Public Health Service (Anderson, 1956) recommended pasteurization of milk at 145°F for 30 minutes by the vat method, or at 161°F for 15 seconds by the high-temperature short-time method, is adequate to protect the public health.

Pasteurization of milk, as it is employed today, is relied upon to destroy pathogenic microorganisms which might be present. It is not intended to destroy all microbial life and many types of organisms persist in the finished product. The application of pasteurization processes to beverages other than milk is usually intended to destroy a certain group of heat-labile organisms responsible for characteristic spoilage of the product in question. Recently, however, in the case of salmonellae in liquid egg, pasteurization has again been employed to destroy pathogenic organisms in food (Winter, et al., 1946; Gibbons, et al., 1946; Gorseline, et al., 1951).

CANNING

The problems involved in the thermal processing of canned food-stuffs are different from those related to the production of pasteurized products. In canning, "commercial sterilization" is the condition sought. This is a state in which the spoilage and pathogenic types of organisms have been destroyed by heat and only the spores of some highly heat-resistant forms remain viable. However, these forms do not develop during storage or cause undesirable changes in the canned product.

Though the canning industry rapidly expanded after Appert's discovery, it was not until late in the 19th century that the importance of heat penetration was recognized and investigations of the phenomenon

were conducted. Early reference to this subject was made by Prescott and Underwood in 1898 (Ball 1938). By the use of maximum registering thermometers, they found that the center of a can of corn, being processed in a retort at 245.8°F, was 226.9°F after 30 minutes exposure, 237.2°F after 45 minutes and 245.8°F after 55 minutes. Once the idea of heat penetration was established it was not long before the concept of time-temperature relationships for the death-points of spores was understood and the development of commercial thermal processes developed rapidly. Through the efforts of Bigelow, *et al.* (1920), Ball (1923, 1927, 1928), and Olson and Stevens (1939) calculation methods for determining process times and temperatures are now available and thermally processed canned goods represent one of the safest and most stable forms of food available.

The only pathogenic organism known to cause spoilage in canned goods is Clostridium botulinum, since other pathogens are destroyed by the heating process. This organism produces a heat resistant spore and a highly potent and lethal extracellular toxin. The fundamental knowledge of the heat resistance of bacteria has been applied to C. botulinum and canners have acquired accurate information on the temperature and length of time necessary to safeguard food products from botulism (Esty and Meyer, 1922; Esty, 1923; and Williams, 1924). As a result of the application of this information in a practical manner, foods canned commercially in the United States have not been implicated in botulism since 1925, with a few minor exceptions involving small outbreaks among individuals who have eaten specialty items, such as mushroom sauce (Tanner, 1953).

REFRIGERATION

In the past, comparatively little attention has been given to the effects of low temperatures on bacteria. Presumably, this is so because of the general belief that cold represents an excellent means of preventing putrefaction and decay. Though the use of low temperatures for food preservation did not become prevalent until the nineteenth century, the preservative effects of cold had long been recognized by the primitive peoples of Northern Europe.

Ice was not used to any considerable extent for commercial refrigeration of food before 1830, though some lots of ice-packed fish were shipped in the United States via the Erie Canal prior to this time. Between 1830 and 1860 the use of ice for preservation was given impetus through the development of commercial methods for harvesting natural ice and the invention of the ice-cooled refrigerator. In the years 1860 to 1890, natural-ice refrigeration represented real progress in the solution of food-supply problems for the growing urban population of the United States. However, by the turn of the century, because of the cost of transporting natural ice to the warm areas of the country and the contamination often associated with it, many legitimate objections were raised

to its use in the food industry and particularly for table service (Anderson 1953).

The development of the mechanical refrigeration industry was a logical outgrowth of natural ice refrigeration, in view of the objections raised to the latter and the dependency of its supply on the vagaries of the weather. Early inventors and engineers concentrated on developing methods for producing ice artificially and an excellent review of the history of the development of these machines has been compiled by Nickerson (1915). In the decade 1880 to 1890, great progress was made in the development of individual machines uniquely suited for application in the meat-packing and fermentation industries. However, because of the absence of means for exchanging technical information, little, if any, thought was given to applying mechanical refrigeration to other areas.

It was at this point, the winter of 1890, that impetus was given to the development of the mechanical refrigeration industry by the failure of the natural ice crop. Though the shortage was disastrous, it awakened the public to the use of mechanical refrigeration.

Because of the demand for ice placed on the few manufacturers operating on a commercial basis in 1890, unification and cooperation between them became imperative. From this beginning grew a coordinated refrigeration industry which rapidly improved methods for commercially manufacturing ice. With the availability of large quantities and a constant supply of ice, the ice-cooled home refrigerator appeared and gained in popularity. Gradually, however, again through the leadership of the meat-packing and fermentation industries, artificial refrigeration replaced ice refrigeration. The engineering accomplishments of this transitional period are not pertinent to this review; however, the historical development of artificial refrigeration and the contributions of the many individuals responsible for its growth have been reviewed by Goosman (1924) and Anderson (1953).

BACTERIOSTATIC EFFECT OF SUBFREEZING TEMPERATURE

The fact that refrigerated storage increased the shelf life of foods was evidence that cold repressed the microbial activities responsible for food spoilage. However, early research of the effects of low temperature on bacteria revealed that cold had little germicidal activity. Macfadyen (1900) exposed cultures of Bacterium coli, S. typhi, Bacillus anthracis, Vibrio cholerae, Proteus vulgaris, and Staph aureus for 20 hours to liquid air at temperatures of -259.6°F to -310°F . Upon subculture, the organisms grew well and maintained their usual biochemical properties. Paul and Prall (1907) exposed staphylococci to liquid air and found that they retained their viability for several months. Haines (1938) reported that rapid freezing with solid carbon dioxide at -94°F killed a high proportion of some organisms but had little effect on others.

As early as 1927 Damon and Leiter reported on the extent of growth and physical changes produced by Bacillus suispestifer, Bacillus pestiscaviae (typhimurium), Bacillus sanguinarum (gallinarum) and Bacillus anatum in foods stored at ice box, 50°-59°F; room, 68°-72°F; and body temperatures, 98.6°F. The organisms grew luxuriantly in various meats, fish, vegetables, fruits, puddings, and milks at the higher temperatures and, with but a few exceptions, also grew extremely well at the ice box temperatures of 50°-59°F. Prescott and Geer (1936) and Prescott and Tanner (1938) claimed that temperatures of 41°F or below were required to totally prevent the growth of salmonellae in foods.

Evidence is also available that temperatures of less than 50°F are necessary to prevent the development of staphylococci in foods. Kelly and Dack (1936) reported slow growth of enterotoxigenic staphylococci in ham, tongue, and chicken sandwiches held at 46°F, and Jones and Lockhead (1939), reporting on micrococci which survived in frozen-pack vegetables, showed that growth of enterotoxic staphylococci was inhibited at 40°F. In view of early observations that temperatures of less than 50°F were required to prevent the development of salmonellae and staphylococci in foods, it is surprising that so little attention has been paid to this fact. The present acceptance of 50°F as the maximum tolerable limit for refrigerating foods is probably related to the inability of Cl. botulinum to produce toxin at this temperature and to the inability of the older ice-cooled refrigerators to maintain temperatures much below 50°F. In addition, storage of perishable foods at this temperature for short periods of time did not give rise to food-poisoning episodes. However, based on the information available, this temperature is not adequate to prevent the growth of salmonellae and staphylococci when held in foods for extended periods. Though 50°F is adequate for short holding intervals, extended storage at this temperature often results in dangerous increases in their numbers.

PART ONE. ORIENTATION EXPERIMENTS WITH BROTH CULTURES

PURPOSE

These experiments were performed to determine the response of several enterotoxigenic staphylococci and food-poisoning salmonellae in brain heart infusion broth to temperatures ranging from 41°F to 158°F; to orient the investigators as to the temperature limits at which growth, survival, or death of the test strains occurred; to aid in selecting a few strains of both types of organisms with characteristic temperature responses; and to define the temperatures and sampling intervals suitable for subsequent studies in foods.

METHODS

The following antigenically distinct species of *Salmonella*, representative of those often associated with foodborne salmonellosis, were selected for use in the broth studies: *S. manhattan*, *S. enteritidis*, *S. typhimurium*, *S. senftenberg*, *S. cubana*, *S. montevideo*, and heat resistant strains of *S. typhimurium* 6087 and of *S. senftenberg* 775W.

Eight enterotoxigenic strains of *Staphylococcus aureus*, each with a distinctive phage pattern, also were employed. The strains were designated as: Ms 149, 21 B, 196 E, MF 159, 224, 2Y, D 13, and 161 C. All test organisms were maintained on nutrient agar slants incubated at 95°F for 18 to 24 hours. To prepare an inoculum for a given experiment, the growth from an 18-hour slant culture was washed into suspension in sterile phosphate buffered dilution water (Am. Pub. Health Assoc. 1953). *Salmonella* suspensions were adjusted to 60% transmittance and the staphylococci suspensions to 45% transmittance at 620 millimicrons on a Coleman Junior Spectrophotometer. These turbidities were equivalent to approximately 1×10^9 viable cells per ml. as determined by platings in Bacto Plate Count agar and incubation at 95°F for 24 hours. One ml. aliquots of the prepared suspensions were added to duplicate tubes, each containing 10 ml. of sterile Bacto brain heart infusion broth and pre-adjusted to the desired test temperature. The inoculated tubes were mixed thoroughly, zero time turbidity readings obtained, and the cultures incubated in water baths at the test temperatures. To determine the actual number of organisms added to the broth, two control tubes at room temperature were inoculated, mixed identically to those in the test, immediately plated in duplicate in Bacto Plate Count agar, and the plates incubated at 95°F for 24 hours. At various intervals, depending upon the temperature, turbidity measurements were made on the inoculated broth tubes in the test series.

In addition to turbidimetric determinations, plate counts also were made at selected intervals on aliquots of the salmonellae and staphylococci broth cultures in order to determine the number of survivors.

The sampling intervals employed were not intended to yield thermal death point determinations, but rather, to give some indication of the temperatures at which growth, survival without multiplication, and death occurred during time intervals approximating those encountered during commercial storage, preparation, or serving of foods.

RESULTS

Staphylococcus aureus

In the case of the staphylococci, no significant increase in numbers of organisms per ml. was observed at 41°F throughout a seven-day incubation period; whereas six out of eight strains grew to some extent at 50°F (Table A1). A five- to eight-fold increase in numbers of viable cells per ml. occurred at 59°F after 72 hours; and an eight- to twelve-fold increase after 48 hours at 68°F (Table A2). The differences in growth response were related to differences in strains. With the exception of strain MF 159, good growth of all the staphylococcal cultures occurred at 113°F and the maximum cellular concentrations were reached between four to eight hours' incubation (Table A3). Though multiplication of MF 159 was observed, growth was not as luxuriant as that of the other strains.

At 122°F no growth of any of the test strains occurred. Apparently, the critical temperature at which multiplication was no longer possible under the test conditions fell between 113°F and 122°F. Upon prolonged incubation at 122°F, a gradual decrease in numbers was noted and the cultures approached sterility by the end of 24 hours (Table A4).

Survival at temperatures between 131°F and 158°F also varied according to strain. On the basis of survival data obtained at these temperatures, minor differences in heat resistance were observed. Strain Ms 149 survived 158°F for 10 minutes, strain 196E survived 158°F for 5 minutes and strain MF 159 survived 149°F for 15 minutes, but not 158°F for 10 minutes (Tables A5 through A8).

Salmonellae

In the case of the salmonellae, the response to temperature was similar to that observed for staphylococci, though, as a group, they did not survive high temperatures as well. No change in numbers occurred at 41°F through a 72-hour period. However, a three- to twelve-fold increase in numbers of viable cells per ml. occurred among the test organisms at 50°F after 72 hours (Table A8); a seven- to twelve-fold increase at 59°F

after 24 hours (Table A9); and a ten- to twenty-fold increase at 68°F after 24 hours (Table A10). The differences were species related.

Good growth of all the test species occurred at 113°F and the maximum stationary phase of growth was reached between four and eight hours' incubation (Table A11). No growth of any of the test species occurred at 122°F and no detectable survivors were present after 24 hours (Table A12). As with the staphylococci, the temperature at which multiplication no longer was possible fell between 113°F and 122°F. By determining the number of survivors present at various intervals for temperatures of 112°F to 140°F, it was possible to estimate differences in heat resistance among the test species.

On the basis of these differences, the following organisms were selected as representative of high, medium, and low heat resistance, respectively: S. senftenberg 775W, S. enteritidis, and S. manhattan. S. senftenberg 775W survived 15 minutes' exposure at 140°F (Table A16), S. enteritidis survived 15 minutes' exposure at 131°F (Tables A13 and A15), and S. manhattan survived 3 hours' exposure at 122°F (Table A12), but did not survive 15 minutes' exposure at 131°F (Tables A13 and A15).

DISCUSSION

Based on their reactions in brain heart infusion broth, it was found that staphylococci and salmonellae generally displayed similar responses to temperature. At a refrigerator temperature of 41°F, neither type of organism grew. However, at 50°F, significant multiplication of all the salmonellae cultures occurred within 24 hours and six out of eight strains of staphylococci displayed some multiplication within 72 hours.

These findings become important when it is realized that many new types of prepared and ready-to-serve foods are subjected to much longer periods of refrigerated storage than were necessary with foods available at the time that 50°F was accepted as the upper limit of refrigeration. Because of this, more precise information is needed on the growth responses of food-poisoning microorganisms at temperatures of 40°F to 50°F. If multiplication of foodborne pathogens occurs in foods stored at 50°F to the same extent as that observed in broth, hazardous increases of these organisms may be expected during prolonged storage.

Another similarity in temperature response between the two groups of organisms was that multiplication apparently ceased at temperatures between 113°F and 122°F. However, above 122°F, survival rates of salmonellae and staphylococci varied considerably. For the most part, the staphylococci survived at higher temperatures and for longer periods than did the salmonellae; a fact which emphasizes that recommendations for holding foods at lethal temperatures should be based upon the survival rates of the most heat-resistant types of the infectious or toxigenic organisms likely to be encountered in the food.

The results obtained in broth on the effects of high temperatures upon the test strains indicated that similar research employing selected types of foods often involved or incriminated in food-poisoning outbreaks is needed to determine precisely the temperature at which multiplication ceases. In addition, they indicated that holding temperatures of 140°F and 150°F may be much greater than actually required to kill pathogens in foods being held under conventional conditions of serving and dispensing. To properly evaluate this point, further studies conducted with foods are necessary. Though reductions in storage and dispensing temperatures usually result in improved palatability and consumer acceptance, such reductions are warranted only after adequate evidence is gathered that reduced holding temperatures provide the necessary public health safeguards.

SUMMARY

Studies were conducted on the growth and survival of several food-poisoning salmonellae and staphylococci in brain heart infusion broth to determine their response to temperatures ranging from 41°F to 158°F.

The results indicated that neither group of organisms multiplied at 41°F, and that good growth of the salmonellae occurred at 50°F, but only fair growth was observed on the part of six of eight staphylococcal strains at this temperature. Good growth of both groups was also noted at 59°F and 68°F.

At 113°F excellent growth of all the test cultures, except one strain of staphylococcus, was noted and the maximum cellular concentrations were attained between four and eight hours' incubation.

No growth of any of the test cultures occurred at or above 122°F; however, the staphylococci survived at higher temperatures and for longer time intervals than the salmonellae.

Based on their reaction to temperatures, the following cultures were selected as showing varying heat resistance and ability to survive cold temperatures: S. senftenberg 775W, S. enteritidis, and S. manhattan, high, medium and low heat resistance respectively. Minor differences in heat resistance were observed among the staphylococci, with Staph. aureus Ms 149 displaying a slightly greater resistance than strains 196E and MF 159.

PART TWO. BEHAVIOR OF SALMONELLAE AND STAPHYLOCOCCI IN
SELECTED FOODS STORED AT TEMPERATURES OF 40°F THROUGH 50°F

PURPOSE

Though numerous investigations have shown that storage of food at 50°F is sufficient to prevent the development of Clostridium botulinum in foods (Edmanson, et al., 1923; Staren, 1926; Tanner and Eglesby, 1936; Tanner, et al., 1940), comparatively little data has been published on the behavior of other foodborne pathogens at this temperature. The recommendation of the Public Health Service is, that perishable foods be maintained at 50°F, or below (Ordinance and Code Regulating Eating and Drinking Establishments, PHS Pub. No. 37; The Vending of Foods and Beverages, a Sanitation Ordinance and Code, PHS Pub. No. 546). Evidence gathered from studies conducted in brain heart infusion broth and presented in Part One of this report, indicate that enterotoxigenic staphylococci and food-poisoning salmonellae multiply well at this temperature. However, these same studies revealed that multiplication did not occur at 40°F. In view of the limited information available concerning the temperature necessary to prevent multiplication of salmonellae and staphylococci in refrigerated foods, it is evident that an organized body of information is needed concerning the growth response of these organisms at low temperatures.

This investigation was undertaken to supply, in part, the necessary information and reports on the growth response of salmonellae and staphylococci at 40°F to 50°F in foods representative of the types associated with food-poisoning outbreaks.

METHODS

Custard, ham salad, and chicken à la king were selected as foods representative of the types often incriminated in cases of food-poisoning. The foods were prepared according to the following recipes:

Custard

	Approx.	
Corn starch	(8 tbsp.)	40 g.
Salt (NaCl)	(1/2 tsp.)	2 g.
Sugar (sucrose)	(1 1/2 cups)	322 g.
Milk	(1 qt.)	946 ml.

Egg yolks, lightly beaten	4
Egg whites, stiffly beaten	4
Vanilla (3 tsp.)	14.7 ml.
pH 6.8	

Mix dry ingredients together. Add one quart milk and four lightly beaten egg yolks. Cook in double boiler until thick. Add stiffly beaten egg whites from four eggs and 3 teaspoons of vanilla and fold in gently.

Ham Salad

	Approx.	
Cooked ham	(1 lb.)	454.0 g.
Sweet pickles	(1/5 cup)	93.5 g.
Cooked pimentos	(1/10 cup)	49.5 g.
Raw green pepper	(1/5 cup)	77.0 g.
Salad dressing	(1/5 cup)	75.0 g.
Water		150.0 ml.
Hard boiled eggs	2	
pH 5.6		

Place all ingredients in hopper of meat grinder and pass through the grinder two times. Add water to ground product and mix thoroughly.

Chicken à la king

	Approx.	
Butter	(3 tbsps.)	40.0 g.
Flour	(3 tbsps.)	30.0 g.
Milk	(2 cups)	473.0 ml.
Cooked chicken	(1 cup)	227.0 g.
Cooked pimentos	(1/2 cup)	113.5 g.
Raw green peppers	(1/4 cup)	113.5 g.
Salt	(1/2 tsp.)	2.0 g.
Black ground pepper	Pinch	.25 g.
Water		75.0 ml.
pH 6.2		

Mix flour, butter, and milk together and heat in double boiler until thick. Place remaining ingredients in hopper of meat grinder and pass through grinder twice. Add ground ingredients to white sauce, add water, and mix thoroughly.

Following their preparation, the three types of foods were weighed out in 50 g. aliquots and the aliquots placed in 2 oz. screw-cap jars. After filling, the jars were tightly closed and the foods were cooked and sterilized as follows: ham salad and chicken à la king at 252°F for 15 minutes, custard at 239°F for 15 minutes. The pH of each of the foods

after cooking was 5.6, 6.2, and 6.6, respectively. After cooling, the screw caps on each of the jars were covered with 5" x 5" squares of "dental dam" rubber sheeting* held in place by means of a rubber band wound tightly over the sheeting, but below the bottom thread of the jar. This procedure prevented seepage of water into the jars. The jars of food were then totally submerged in each of a series of constant temperature water baths previously adjusted by means of a Bureau of Standards thermometer. The baths maintained the following temperatures plus or minus 0.9°F; 95°F, 50°F, 48°F, 46°F, 44°F, 42°F, and 40°F. Dual scale centigrade-fahrenheit thermometers, checked for accuracy against the Bureau of Standards thermometer, were maintained in the baths throughout the experiments.

Based on the results obtained from the broth studies cited in Part One of this report, the following organisms were selected as inocula: S.senftenberg 775W, S.enteritidis, S.manhattan, and Staph.aureus 196 E, Staph.aureus MF 159, and Staph.aureus Ms 149. Stock cultures of the organisms were maintained by daily transfer on nutrient agar slants incubated at 95°F. To prepare the inocula for a given experiment, the growth from a 24-hour slant was washed off with sterile phosphate buffered distilled water (Am. Pub. Health Assoc. 1953), shaken vigorously in a sterile colorimeter tube to break up clumps and adjusted to the desired concentration. The salmonellae suspensions were adjusted to 18% transmittance at 620 millimicrons on a Coleman Junior Spectrophotometer and the staphylococcal suspensions to 10% transmittance. Following adjustment, the three salmonellae suspensions were pooled together to make a mixed culture of the three species. The staphylococci were similarly pooled to form a second mixed culture. Each mixed culture yielded approximately 5,000,000,000 viable cells per ml. by plate count. Mixed cultures were used for inocula, because it was felt that suspensions containing organisms of varying heat resistance would represent more closely the response to temperature of salmonellae and staphylococci in general than that possible by the use of pure cultures. In addition, the use of multiple strains minimized the importance to the final result of a given food affording a single strain an unknown substrate specificity which might affect its response to temperature.

One-tenth-ml. aliquots of each mixed-culture suspension were inoculated into duplicate jars of each of the three foods adjusted to the desired test temperatures. This volume of suspension, added to the 50 g. of food in each jar, resulted in a final inoculum of approximately 10,000,000 cells per gram. After inoculation, the screw caps were replaced and the jars were again covered with "dental dam." The inoculated jars were then shaken vigorously by hand for 50 return strokes to distribute the inoculum throughout the mass of food. After shaking, the jars were once again totally submerged in the water baths and permitted to incubate for 5 days. At 24-hour intervals, plate counts of the inoculated foods were performed by

*Hygienic dental dam, half-yard length, 5-inch width, medium grade, manufactured by: The Hygienic Dental Manufacturing Company, Akron 10, Ohio.

removing 10 g. of food and grinding it in a sterile Waring Blendor cup with 90 ml. of sterile distilled water for 2 minutes at slow speed and preparing appropriate 10-fold dilutions from these blends in sterile phosphate buffered distilled water (Am. Pub. Health Assoc., 1953). Duplicate plates of each dilution were poured, employing Bacto Plate Count agar, and the plates were incubated 24 hours at 95°F.

One uninoculated jar of food also was incubated at each of the temperatures to serve as a "leak" control. These jars were sampled and plated similarly to the test jars.

To determine that the heat treatment employed was sufficient to sterilize the foods, individual jars of each were analyzed as follows: the total 50 grams of food from a single jar was dropped into 430 ml. of sterile distilled water in a Waring Blendor cup. The jar was rinsed with two additional sterile 10 ml. rinses. These were also added to the cup. The resultant 1-10 dilution was blended 2 minutes at slow speed. A 10-ml. aliquot of the blend was distributed among three Petri plates which were poured with Bacto Plate Count Agar. The poured plates were incubated at 95°F for 24 hours.

To determine quantitatively the number of organisms per gram initially present in the inoculated food samples, two additional jars of each food were inoculated with salmonellae and two with staphylococci. After shaking, the contents of each were dropped into a Waring Blendor cup containing 430 ml. of sterile distilled water. The jars were rinsed with two 10 ml. rinses which were also added to the cup. The resultant 1-10 dilution was blended 2 minutes at slow speed and plated as described for the test series.

RESULTS

Significant differences in growth response between the salmonellae and staphylococci, as related to temperature and food menstruum, were observed. These differences are shown in Figures 1 through 3 of the text, which represent the individual growth curves of both groups of organisms at the various incubation temperatures and in the three food products. Each point on these curves represents the mean of duplicate plate counts obtained from each of the paired jars of food inoculated with the same culture and held at a given temperature. For detailed observations of the individual plate count values obtained in each of the foods for the two groups of organisms, reference should be made to Tables B1 through B3 of Appendix B.

In cream custard (Figure 1 and Table B1) the staphylococci showed no increase in numbers throughout 5 days of incubation at 40°F and 42°F, but a slight increase on the 5th day at 44°F. However, an increase was observed at the end of 4 days' incubation at 46°F and 48°F; and 2 days' incubation at 50°F with progressive increases throughout the remainder of the 5-day period at these temperatures.

The results shown in Figure 1 indicate that the slow decline in numbers of salmonellae was related to the inability of these organisms to reproduce in custard at the low test temperatures, and not to an inhibitory property of the custard per se, for good growth of both salmonellae and staphylococci occurred in custard at 95°F.

In chicken à la king (Figure 2 and Table B2), the growth responses of the salmonellae and staphylococci to temperature again differed from each other. In addition, the temperatures at which growth was initiated and the final cellular concentration per gram differed from the values observed in custard. Neither group of organisms displayed any significant change in numbers of cells per gram throughout the 5-day incubation period at 40°F or 42°F. However, at 44°F, increases were noted in both groups at the end of 4 days' incubation. At the end of the fifth day, increases of approximately 50,000,000 salmonellae and 5,000,000 staphylococci per gram were noted over the numbers present on the fourth day.

At 46°F and 48°F, the salmonellae grew more rapidly than staphylococci and yielded considerably higher cellular concentrations. At 50°F both groups of organisms displayed good growth and the growth of the salmonellae was only slightly better than that of the staphylococci. The maximum stationary phase of growth was reached by both groups after 4 days.

By way of comparison, a concentration of approximately 1,000,000,000 organisms per gram was attained in chicken à la king by both groups at the end of 24 hours' incubation at 95°F; whereas, 4 days' incubation was required to attain similar results at 50°F.

In ham salad (Figure 3 and Table B3), no significant increase in numbers of salmonellae or staphylococci occurred at 40°F through 50°F during 5 days' incubation. At all the low temperatures, the staphylococci underwent initially a slight drop in numbers followed by a gradual return to the level of the original inoculum by the end of 5 days. On the other hand, the salmonellae underwent a gradual continuing decline in numbers throughout the 5 days. At 95°F, however, good growth of both groups was observed.

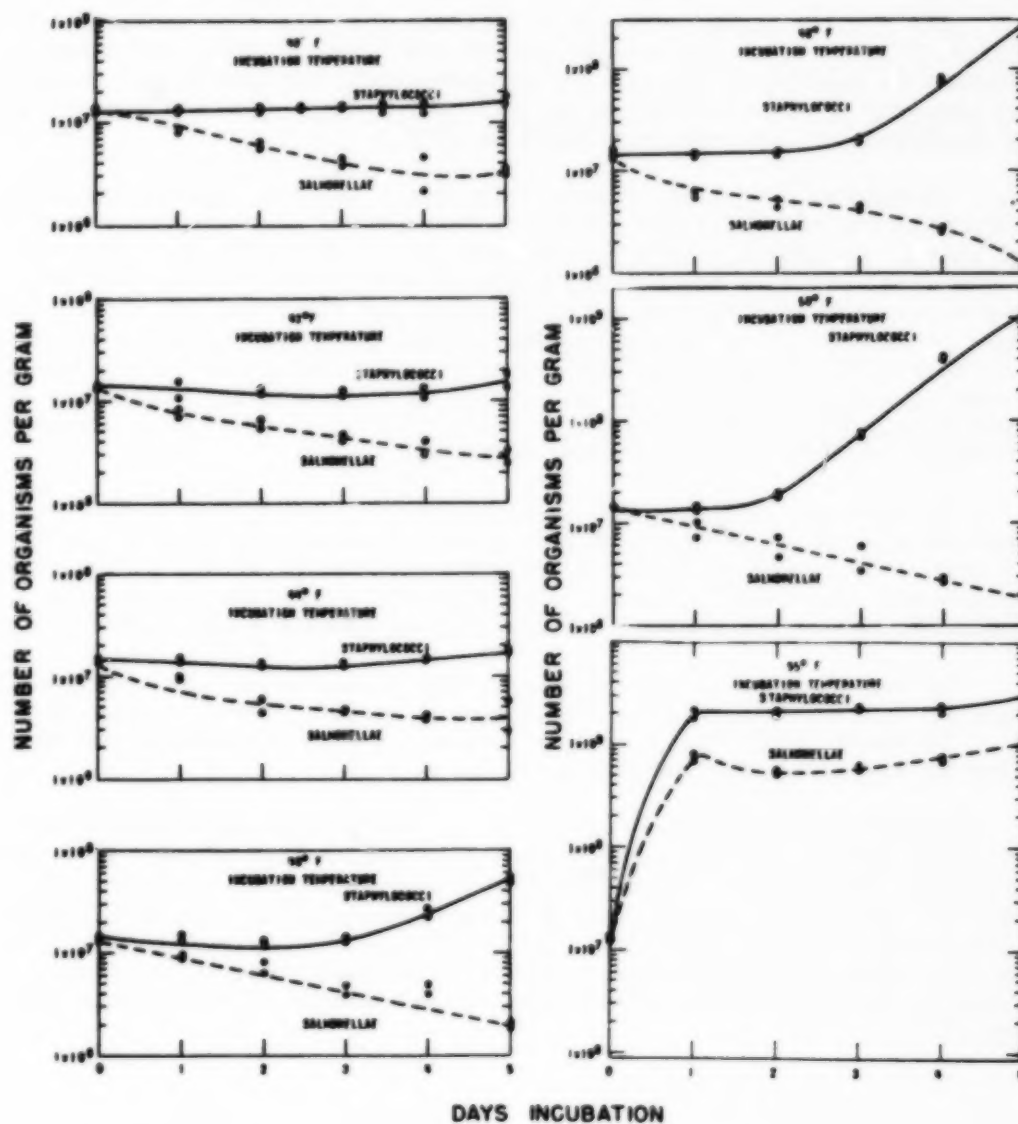


Figure 1. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN CUSTARD INCUBATED AT VARIOUS TEMPERATURES.

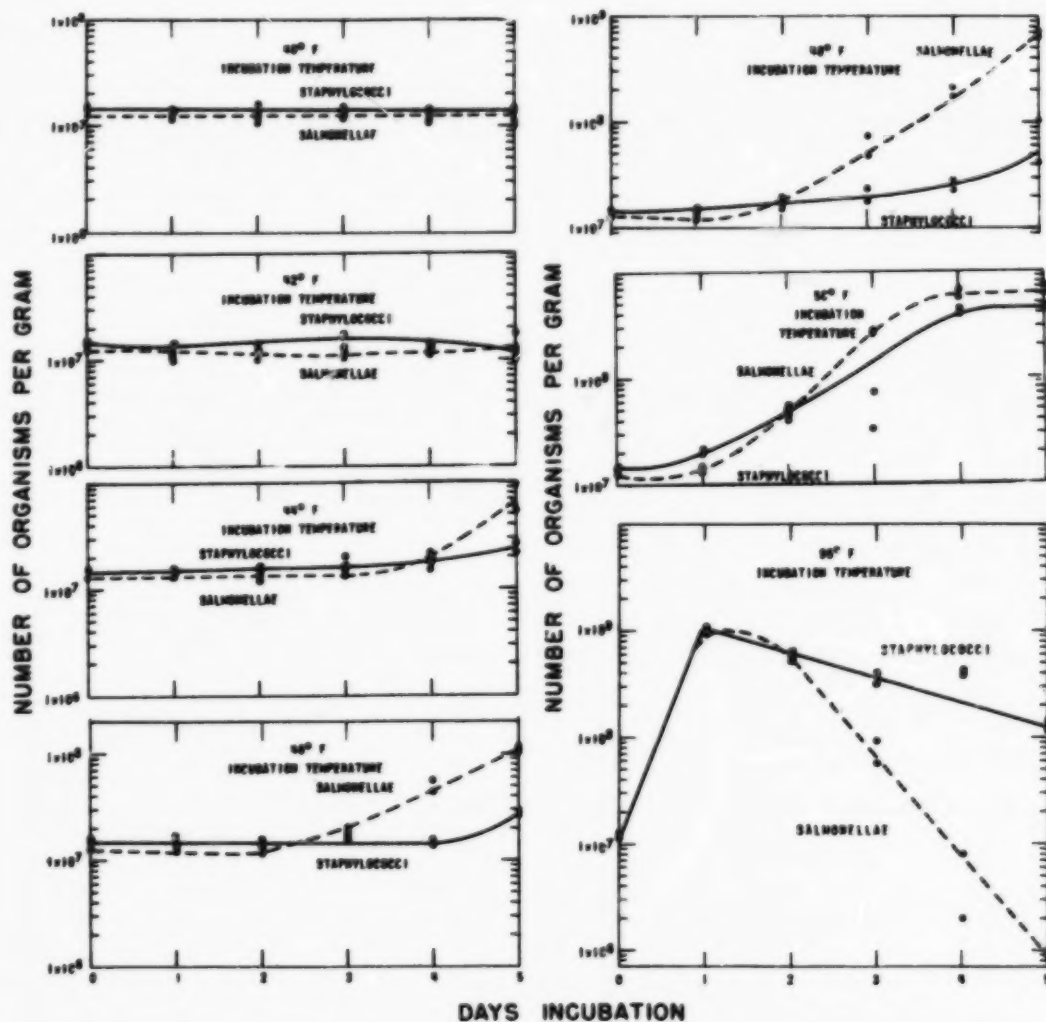


Figure 2. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN CHICKEN A LA KING INCUBATED AT VARIOUS TEMPERATURES.

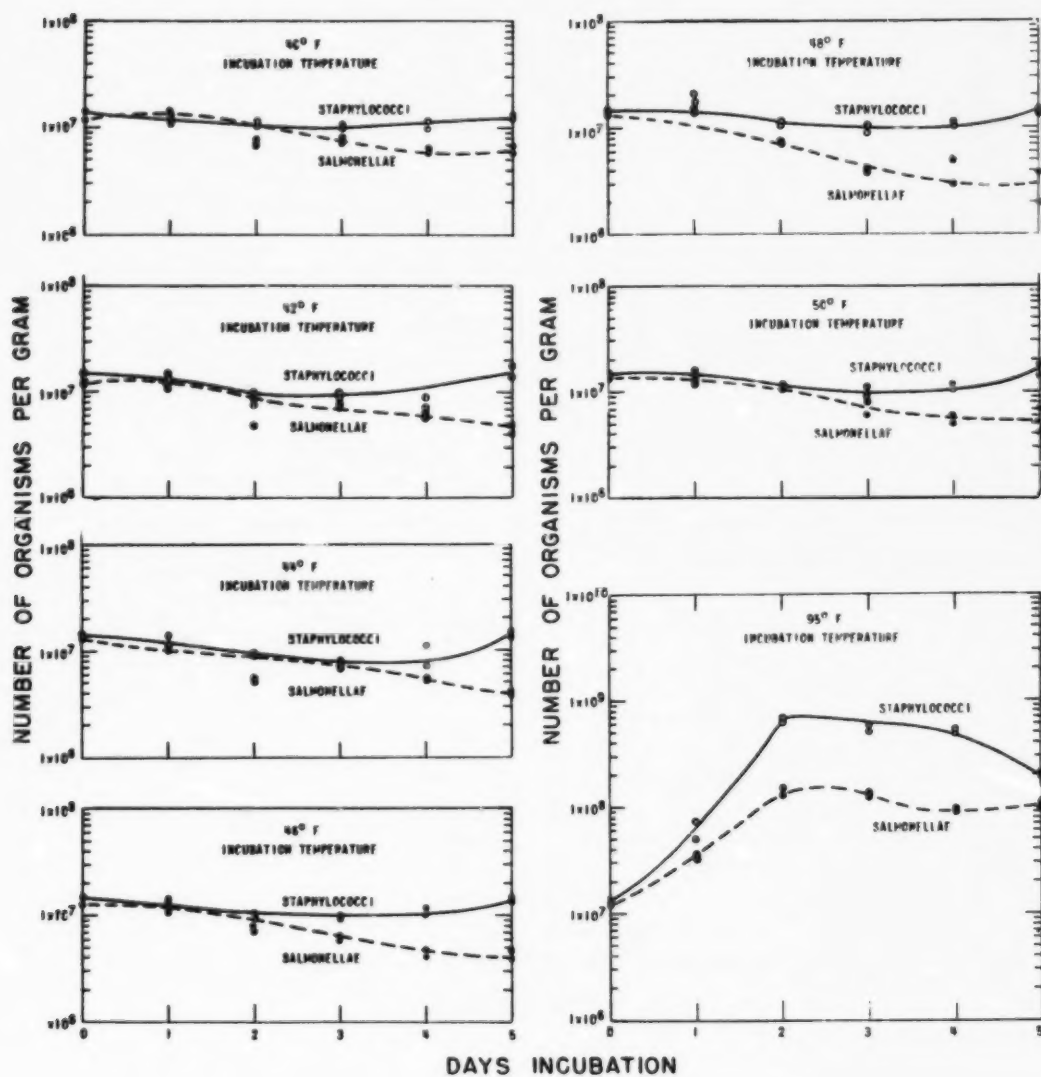


Figure 3. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN HAM SALAD INCUBATED AT VARIOUS TEMPERATURES.

DISCUSSION

Results obtained with salmonellae and staphylococci indicate that the following table provides a useful guide to refrigeration temperatures and times, based on internal food temperature for readily perishable cooked foods of the types used in this study.

Table 1. REFRIGERATION TEMPERATURES PREVENTING THE GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN READILY PERISHABLE COOKED FOODS

Storage Temperature in °F	Days at which foods were stored without growth
50	1
48	2
46	3
44	4
42	5+
40	5+

The storage periods shown for the various temperatures represent the time intervals at which all the foods used in this study were stored without significant multiplication of salmonellae or staphylococci.

It is apparent from the data presented earlier that the practice of storing perishable foods at a temperature of 50°F for more than one day may result in dangerous increases of the microbial populations. Because microbial growth occurs relatively slowly at 50°F, it is possible to store foods safely at this temperature for

short periods, provided the foods are not grossly contaminated with toxigenic or infectious microorganisms. The general adoption of this temperature as the maximum upper limit of refrigeration for perishable foods is probably related to this fact. However, the ability of microorganisms to multiply at 50°F, and the danger that such multiplication will produce microorganisms capable of causing illness cannot be emphasized too strongly. The data collected in this study indicate that if it is desirable to store perishable foods for extended periods, internal temperatures of the food should be maintained at or below 40°F or 42°F in order to prevent growth of salmonellae and staphylococci.

In discussing holding temperatures for perishable foods, it is also important to note the rate at which foods cool with reference to volume and type of container. Though growth of the test organisms at 40°F and 42°F did not occur in food even after 5 days' incubation, it must be recognized that an important contributing factor to this result was that the foods had been adjusted to these temperatures before inoculation and were not permitted to deviate from them throughout the experimental time limit. In practice, the problem of cooling foods to temperatures of 40°F or 42°F becomes important. The object is to take the food as rapidly as possible through the range of temperatures at which microbial growth is possible to one at which growth ceases. The rate at which a food cools is related to the differential in temperature between it and the cold storage area, the type and volume of the food, and the type and shape of container in which it is placed. Though it is recognized that many objections have been raised to placing hot foods directly into the refrigerator without previous

cooling to room temperature, it is fallacious reasoning in the era of mechanical refrigeration to believe that prompt cooling of hot foods causes "souring" or that a "sealing-in" of heat occurs due to the congealing of outer layers. This point of view probably originated when ice boxes were the only source of refrigeration and introduction of hot food caused the ice to melt and elevated the temperature of the box to a point favorable for growth. The work of Black and Lewis (1948) revealed that prompt refrigeration of hot foods resulted in faster internal cooling than did initial cooling to room temperature followed by refrigeration. They found that the time refrigerated foods remained within the temperature growth range for food-poisoning bacteria was lessened by refrigeration of the food before its internal temperature dropped to 115°F. Lewis, *et al.* (1953), presented data correlating food volume and container size with cooling rates and showed that a difference of as much as six hours was necessary to lower the temperature of a large volume of chicken salad in a deep bowl as compared to the same volume distributed among shallow pans.

The formulation of a food also may affect the survival or multiplication of microorganisms therein. For example, the ham salad of the above experiments was maintained for 5 days at 50°F with no over-all increase of either salmonellae or staphylococci. However, had a ham salad been prepared from a different formulation, good growth of the test organisms might have occurred. Food poisoning attributable to ham salad is not uncommon. Googins (1958) reported an outbreak in which 600 high school girls became ill within 1 to 10 hours after eating ham salad sandwiches in the school cafeteria. Coagulase-positive Staphylococcus aureus was isolated from the ham salad in numbers in excess of 1,000,000 organisms per gram. Tartakow (1956) also reported a similar outbreak in which 28 persons became ill within 1 1/2 to 7 1/2 hours after ingesting ham salad, egg salad, or cream cheese sandwiches. Bacteriological examination of several sandwiches revealed the presence of coagulase-positive, hemolytic Staphylococcus aureus. Though information relating the temperatures at which these foods were held is not available, these outbreaks indicate that ham salad does support the growth of food-poisoning bacteria and is a food for which strict sanitary control is necessary during preparation.

In applying the information presented in this study to formulate criteria for safe storage of readily perishable foods, maintenance of refrigeration at 40°F or below seems necessary to allow for unavoidable variation in holding time levels, and types of contamination, volume and kinds of foods, frequency of opening the refrigerator as well as other factors that may be expected to affect every day practice.

Thus, it is evident from the above discussion that a number of factors contribute to safe refrigerated storage of perishable foods. The recipe appears to be important and the formulation of recipes which result in an end-product unfavorable for the growth of bacteria may provide some degree of safety: for example, the incorporation into recipes of ingredients which increase the hydrogen ion concentration to the critical level of pH 4.5 (Tanner, 1948; Banwart and Ayres, 1957) or the addition of cer-

tain amino acids (Castellani, 1953; Castellani, et al., 1955) and preservatives (Chas. Pfizer and Company, Inc. 1955) may be of value. However, until such time as recipes that depend upon formulation to safeguard against the development of food poisoning during storage are accepted for mass feeding operations, prompt and adequate refrigeration appears to be the best safety factor.

SUMMARY

Mixed cultures of S. senftenberg 775W, S. enteritidis, S. manhattan and mixed cultures of Staph. aureus 196E, Staph. aureus MF 159, and Staph. aureus Ms 149 were cultured in custard, chicken à la king and ham salad for 5 days at 2°F intervals from 40°F to 50°F and at 95°F.

In custard, the staphylococci grew at temperatures of 44°F and above. The salmonellae underwent a gradual decrease in numbers at all temperatures from 40°F through 50°F.

In chicken à la king, growth of both salmonellae and staphylococci occurred at temperatures of 44°F and above.

In ham salad, no growth of either group of organisms occurred at 40°F through 50°F.

Good growth of both groups of organisms was observed in all three types of food incubated at 95°F.

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APPENDIX A

Orientation Studies—Effect of temperature on the growth or survival of salmonellae and staphylococci in brain heart infusion broth.

Table 1. EFFECT OF INCUBATION AT 41°F AND 50°F ON THE GROWTH OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Incubation Temperature			
		41°F		50°F	
		Turbidity(a)	No.org/ml ^(b) x 10 ⁻⁶	Turbidity	No.org/ml ^(b) x 10 ⁻⁶
Ms 149	0	94	112	94	112
	24	94	153	94	163
	48	93	129	92	131
	72	92	147	89	128
	96		125		122
	120	92	131	89	106
	168		130		110
21B	0	93	111	93	111
	24	93	154	92	136
	48	93	123	91	105
	72	93	121	89	106
	96		91		93
	120	92	104	87	94
	168		104		95
196E	0	93	65.5	93	65.5
	24	93	71	93	72
	48	93	78	93	76
	72	94	69	92	75
	96		73		69
	120	94	65	92	73
	168		63		71
MF159	0	93	97	93	97
	24	93	121	93	102
	48	93	100	90	94
	72	92	84	88	79
	96		92		79
	120	93	80	84	87
	168		74		131
224	0	93	130	93	130
	24	93	135	92	142
	48	93	138	91	147
	72	93	149	88	106
	96		154		126
	120	93	148	86	184
	168		148		210

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 1. (Continued)

Organism	Hours of Incubation	Incubation Temperature			
		41°F		50°F	
		Turbidity ^(a)	No.org/ml ^(b) x 10 ⁻⁶	Turbidity	No.org/ml ^(b) x 10 ⁻⁶
2Y	0	93	136	93	136
	24	93	154	92	147
	48	94	144	89	133
	72	93	142	87	124
	96		126		119
	120	92	128	88	104
	168		108		101
D13	0	93	137	93	137
	24	93	124	93	120
	48	93	121	94	125
	72	94	107	92	100
	96		97		90
	120	95	101	91	80
	168		100		90
161C	0	93	99	93	99
	24	93	127	92	133
	48	93	124	88	101
	72	92	107	86	86
	96		101		72
	120	92	95	83	72
	168		87		80

(a) Per cent transmittance at a wavelength of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 2. EFFECT OF INCUBATION AT 59°F AND 68°F ON THE GROWTH OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Incubation Temperature			
		59°F		68°F	
		Turbidity(a)	No.org/ml(b) x 10 ⁻⁶	Turbidity	No.org/ml(b) x 10 ⁻⁶
Ms 149	0	93	119	93	119
	24	87	143	66	720
	48	72	560	50	1250
	72	72	710	44	1220
	120				
21B	0	94	126	94	126
	24	86	146	64	990
	48	78	510	52	1080
	72	80	650	46	1170
	120				
196E	0	93	67	91	67
	24	85	96	54	500
	48	69	154	40	790
	72	57	425	36	870
	120		490		
MF 159	0	92	111	92	111
	24	85	130	79	134
	48	72	325	56	500
	72	55	400	50	540
	120		640		745
224	0	92	103	92	103
	24	86	144	64	700
	48	73	480	46	1300
	72	64	750	41	1280
	120				
2Y	0	91	157	91	157
	24	82	221	59	820
	48	71	578	47	1320
	72	71	700	41	1030
	120		720		

- (a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 2. (Continued)

Organism	Hours of Incubation	Incubation Temperature			
		59°F		68°F	
		Turbidity(a)	No.org/ml ^(b) x 10 ⁻⁶	Turbidity	No.org/ml ^(b) x 10 ⁻⁶
D13	0	73	136	92	136
	24	83	149	61	660
	48	71	437	51	1090
	72	70	750	42	975
	120		845		
161C	0	94	94	94	94
	24	85	128	59	770
	48	71	467	39	1150
	72		750	34	955
	120		980		

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 3. EFFECT OF INCUBATION AT 113°F ON THE GROWTH OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	Organism	Hours of Incubation	Turbidity(a)
Ms 149	0	94	224	0	93
	2	80		2	82
	4	48		4	58
	6	38		6	41
	8	40		8	43
	10	40		10	42
	25	40		25	43
21B	0	94	2Y	0	93
	2	81		2	83
	4	50		4	55
	6	46		6	49
	8	46		8	49
	10	47		10	50
	25	50		25	53
196E	0	94	D13	0	94
	2	84		2	80
	4	68		4	52
	6	66		6	49
	8	63		8	50
	10	62		10	50
	25	64		25	53
MF159	0	94	161C	0	94
	2	94		2	80
	4	89		4	61
	6	89		6	52
	8	89		8	53
	10	88		10	53
	25	86		25	59

(a) Per cent transmittance at a wave length of 620 millimicrons.

Table 4. EFFECT OF INCUBATION AT 122°F ON SURVIVAL OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b)
Ms 149	0	94	134,000,000
	5	90	92,500,000
	10	89	7,800,000
	14		52,000
	20		<1
21B	0	94	131,000,000
	5	93	5,400,000
	10	93	217,500
	14		8,150
	20		49
	26		3
196E	0	94	70,000,000
	5	94	154,000
	10	94	3,800
	14		26
	20		<1
MF159	0	94	85,000,000
	5	96	5,300,000
	10	96	172,000
	14		8,300
	20		510
	26		334
224	0	93	101,000,000
	5	92	405,000
	10	93	5,850
	14		118
	20		<1
2Y	0	93	114,000,000
	5	92	87,000,000
	10	93	1,510,000
	14		9,900
	20		62
	26		11

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 4. (Continued)

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b)
D13	0	94	134,000,000
	5	91	101,000,000
	10	92	1,085,000
	14		4,850
	20		3
	26		2
161C	0	94	108,000,000
	5	91	770,000
	10	91	4,050
	14		840
	20		1
	26		<1

(a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 5. EFFECT OF INCUBATION AT 131°F ON SURVIVAL OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	No. of survivors per ml following exposure of:(a)		
	0 hr.	2 hr.	4 hr.
Ms 149	99,000,000	1,000,000	6,700
21B	95,500,000	14,800	102
196E	54,500,000	530	2
MF 159	93,000,000	30	<1
D13	120,000,000	216,000	96
161C	125,500,000	11,500	199

(a) Mean value of duplicate plate counts.

Table 6. EFFECT OF INCUBATION AT 140°F AND 149°F ON SURVIVAL OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	No. of survivors per ml. following exposure of: (a)					
	0 min.	15 min.	30 min.	45 min.	60 min.	90 min.
<u>140°F</u>						
Ms 149	140,000,000	<300,000	1,950	81	34	<1
196E	69,000,000	3,700	23	3	2	<1
MF 159	117,000,000	3,300	185	25	1	<1
<u>149°F</u>						
Ms 149	120,000,000	130	Unsat.	<1		
	124,000,000	1	<1	<1		
196E	92,000,000	1	<1	<1		
MF159	87,500,000	84	<1	<1		

(a) Mean value of duplicate plate counts.

Table 7. EFFECT OF INCUBATION AT 158°F ON SURVIVAL OF STAPHYLOCOCCI IN BRAIN HEART INFUSION BROTH

Organism	No. of survivors per ml. following exposure of: (a)			
	0 min.	5 min.	10 min.	15 min.
Ms149	162,500,000	20,000	110	<1
196E	88,000,000	160	<1	<1
MF159	106,000,000	3	<1	<1

(a) Mean value of duplicate plate counts.

Table 8. EFFECT OF INCUBATION AT 41°F AND 50°F ON THE GROWTH OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Incubation Temperature			
		41°F		50°F	
		Turbidity (a)	No.org/ml (b) x 10 ⁻⁶	Turbidity	No.org/ml (b) x 10 ⁻⁶
<u>S.typhimurium</u>					
	6087 0	97	116.5	96	59.5
	24	97		89	
	48	97		63	
	72	94	108.75	61	745
<u>S.senftenberg</u>					
	775W 0	98	101.5	96	84
	24	97		90	
	48	97		67	
	72	94	90.25	61	800
<u>S.enteritidis</u>					
	0	97	71.0	95	110.5
	24	97		91	
	48	97		73	
	72	95	64.24	66	325
<u>S.typhimurium</u>					
	0	97	102.5	96	121.5
	24	97		92	
	48	97		75	
	72	94	103.5	69	835
<u>S.senftenberg</u>					
	0	98	100.5	96	89
	24	97		91	
	48	97		70	
	72	95	105.75	64	790
<u>S.cubana</u>					
	0	95	83.5	96	72.5
	24	95		91	
	48	97		70	
	72	94	79.75	66	545

(a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

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Table 8. (Continued)

Organism	Hours of Incubation	Incubation Temperature			
		41°F		50°F	
		Turbidity(a)	No.org/ml ^(b) x 10 ⁻⁶	Turbidity	No.org/ml ^(b) x 10 ⁻⁶
<u>S.gaminara</u>	0	95	81.0	97	70.5
	24	95		94	
	48	97		80	
	72	94	86.75	65	645
<u>S.urbana</u>	0	95	106.0	96	73.5
	24	95		92	
	48	96		73	
	72	93	93.5	67	570
<u>S.manhattan</u>	0	95	104.0	96	83
	24	95		92	
	48	97		72	
	72	94	106.0	65	615
<u>S.montevideo</u>	0	95	111.0	96	52.5
	24	96		93	
	48	96		75	
	72	92	96.25	66	610

- (a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 9. EFFECT OF INCUBATION AT 59°F ON THE GROWTH OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) $\times 10^{-6}$
<u>S.typhimurium</u> 6087	0	96.5	83.5
	6	91.5	
	12	72.5	
	24	52.5	
<u>S.senftenberg</u> 775W	0	95.5	99.5
	6	90.5	
	12	74.0	
	24	52.5	
<u>S.enteritidis</u>	0	94.5	121
	6	89.5	
	12	72.0	
	24	53.5	
<u>S.typhimurium</u>	0	94.5	101.5
	6	91.5	
	12	81.0	
	24	58.0	
<u>S.senftenberg</u>	0	95.0	118
	6	89.5	
	12	72.5	
	24	53.0	
<u>S.cubana</u>	0	95.0	100.5
	6	90.5	
	12	71.5	
	24	54.5	
<u>S.gaminara</u>	0	93.5	97.5
	6	92.5	
	12	84.5	
	24	52.5	
<u>S.urbana</u>	0	93.5	77.5
	6	91.0	
	12	77.0	
	24	58.5	
			560

(a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 9. (Continued)

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) $\times 10^{-6}$
<u>S. manhattan</u>	0	94.0	93.5
	6	90.0	
	12	70.5	
	24	52.5	985
<u>S. montevideo</u>	0	94.0	60
	6	91.0	
	12	72.5	
	24	52.0	770

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 10. EFFECT OF INCUBATION AT 68°F ON THE GROWTH OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity ^(a)	No. org/ml ^(b) x 10 ⁻⁶
<u>S. typhimurium</u> 6087	0	95.5	81.5
	4	89.5	
	8	72.5	
	24	46.0	99
<u>S. senftenberg</u> 775W	0	95.0	81.5
	4	86.0	
	8	71.0	
	24	46.5	98
<u>S. enteritidis</u>	0	95.0	88.5
	4	83.5	
	8	71.5	
	24	47.5	1140
<u>S. typhimurium</u>	0	95.5	99
	4	88.0	
	8	78.5	
	24	50.0	1235
<u>S. senftenberg</u>	0	95.5	104.5
	4	85.5	
	8	73.5	
	24	44.5	1225
<u>S. cubana</u>	0	96.5	84.5
	4	90.0	
	8	73.0	
	24	54.5	815
<u>S. gaminara</u>	0	97.5	70
	4	91.5	
	8	80.0	
	24	50.0	1190
<u>S. urbana</u>	0	96.5	47.5
	4	89.5	
	8	69.5	
	24	54.5	990

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 10. (Continued)

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) x 10 ⁻⁶
<u>S.manhattan</u>	0	96.5	78
	4	88.0	
	8	64.5	
	24	50.0	
<u>S.montevideo</u>	0	97.0	47
	4	90.0	
	8	71.5	
	24	50.0	

- (a) Per cent transmittance at a wave length of 620 millimicrons.
(b) Mean value of duplicate plate counts.

Table 11. EFFECT OF INCUBATION AT 113°F ON THE GROWTH OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) x 10 ⁻⁶
<u>S. typhimurium</u> 6087	0	83.0	122.5
	2	78.0	
	4	61.5	
	6	61.0	
	8	59.5	
	24	56.5	
<u>S. senftenberg</u> 775W	0	92.0	65
	2	81.5	
	4	63.0	
	6	61.0	
	8	61.5	
	24	60.5	
<u>S. enteritidis</u>	0	87.5	100.5
	2	81.5	
	4	62.5	
	6	62.0	
	8	62.0	
	24	61.0	
<u>S. typhimurium</u>	0	90.0	85
	2	92.0	
	4	76.0	
	6	66.0	
	8	65.0	
	24	64.0	
<u>S. senftenberg</u>	0	86.5	145
	2	75.0	
	4	60.5	
	6	58.0	
	8	59.0	
	24	54.5	
<u>S. cubana</u>	0	88.0	90
	2	78.0	
	4	62.0	
	6	62.0	
	8	60.0	
	24	60.0	

(a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 11. (Continued)

Organism	Hours of Incubation	Turbidity(a)	No. org/ml ^(b) x 10 ⁻⁶
<u>S.gaminara</u>	0	90.0	95
	2	90.0	
	4	85.0	
	6	77.0	28
	8	60.0	
	24	60.0	
<u>S.urbana</u>	0	90.0	101.5
	2	88.0	
	4	85.0	
	6	74.0	36
	8	63.0	
	24	60.0	
<u>S.manhattan</u>	0	89.0	89.5
	2	84.0	
	4	79.0	
	6	72.0	23
	8	68.5	
	24	63.0	
<u>S.montevideo</u>	0	91.0	91
	2	89.5	
	4	81.0	
	6	74.5	21.5
	8	63.0	
	24	62.0	

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 12. EFFECT OF INCUBATION AT 122°F ON THE SURVIVAL OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) x 10 ⁻⁶
<u>S.typhimurium</u> 6087	0	94.0	101.5
	4	94.0	
	8	94.0	
	24	94.0	0
<u>S.senftenberg</u> 775W	0	93.0	155
	4	95.0	
	8	94.0	
	24	93.5	0
<u>S.enteritidis</u>	0	92.0	155
	4	93.5	
	8	93.5	
	24	93.5	0
<u>S.typhimurium</u>	0	96.0	99
	4	95.5	
	8	95.0	
	24	95.5	0
<u>S.senftenberg</u>	0	96.0	102.5
	4	95.0	
	8	95.0	
	24	95.0	0
<u>S.cubana</u>	0	96.5	92
	4	95.5	
	8	95.5	
	24	95.5	0
<u>S.gaminara</u>	0	96.5	87.5
	4	95.0	
	8	95.0	
	24	95.0	0
<u>S.urbana</u>	0	96.0	114
	4	95.0	
	8	95.0	
	24	95.0	0

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 12. (Continued)

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) x 10 ⁻⁶
<u>S.manhattan</u>	0	95.0	92
	4	94.5	
	8	94.0	
	24	94.0	0
<u>S.montevideo</u>	0	96.0	72
	4	95.0	
	8	94.0	
	24	94.0	0

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 13. EFFECT OF INCUBATION AT 131°F ON THE SURVIVAL OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) $\times 10^{-6}$
<u>S. typhimurium</u> 6087	0	92.0	94
	4	93.0	
	8	94.0	
	24	94.0	
			0
<u>S. senftenberg</u> 775W	0	93.0	100.27
	4	94.0	
	8	94.5	
	24	95.0	
			0
<u>S. enteritidis</u>	0	94.0	95
	4	93.5	
	8	93.5	
	24	95.0	
			0
<u>S. typhimurium</u>	0	92.0	115
	4	93.0	
	8	93.0	
	24	92.0	
			0
<u>S. senftenberg</u>	0	94.0	74
	4	93.5	
	8	93.5	
	24	93.0	
			0
<u>S. cubana</u>	0	96	119.5
	4	95.5	
	8	95.5	
	24	95.0	
			0
<u>S. gaminara</u>	0	96.0	105
	4	95.5	
	8	95.0	
	24	95.0	
			0
<u>S. urbana</u>	0	96.0	121.5
	4	95.0	
	8	95.0	
	24	95.0	
			0

(a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 13. (Continued)

Organism	Hours of Incubation	Turbidity(a)	No. org/ml ^(b) x 10 ⁻⁵
<u>S.manhattan</u>	0	96.0	95
	4	95.0	
	8	95.0	
	24	94.0	
<u>S.montevideo</u>	0	96.0	73
	4	95.0	
	8	95.0	
	24	95.0	

- (a) Per cent transmittance at a wave length of 620 millimicrons.
(b) Mean value of duplicate plate counts.

Table 14. EFFECT OF INCUBATION AT 140°F ON THE SURVIVAL OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity(a)	No. org/ml(b) $\times 10^{-6}$
<u>S. typhimurium</u> 6087	0	95.0	90
	4	95.0	
	8	95.0	0
<u>S. senftenberg</u> 775W	0	96.5	91
	4	95.0	
	8	95.0	0
<u>S. enteritidis</u>	0	95.5	102
	4	95.0	
	8	95.0	0
<u>S. typhimurium</u>	0	95.5	104
	4	95.0	
	8	95.0	0
<u>S. senftenberg</u>	0	94.5	115
	4	93.5	
	8	93.5	0
<u>S. cubana</u>	0	95.0	91
	4	95.0	
	8	95.0	0
<u>S. gaminara</u>	0	96.0	96.5
	4	95.0	
	8	95.0	0
<u>S. urbana</u>	0	96.0	97
	4	96.0	
	8	96.0	0
<u>S. manhattan</u>	0	95.5	95
	4	95.0	
	8	95.0	0
<u>S. montevideo</u>	0	95.5	85.5
	4	95.0	
	8	95.0	0

- (a) Per cent transmittance at a wave length of 620 millimicrons.
 (b) Mean value of duplicate plate counts.

Table 14. EFFECT OF INCUBATION AT 140°F ON THE SURVIVAL OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	Hours of Incubation	Turbidity ^(a)	No. org/ml ^(b) x 10 ⁻⁶
<u>S.typhimurium</u> 6087	0	95.0	90
	4	95.0	
	8	95.0	0
<u>S.senftenberg</u> 775W	0	96.5	91
	4	95.0	
	8	95.0	0
<u>S.enteritidis</u>	0	95.5	102
	4	95.0	
	4	95.0	0
<u>S.typhimurium</u>	0	95.5	104
	4	95.0	
	8	95.0	0
<u>S.senftenberg</u>	0	94.5	115
	4	93.5	
	8	93.5	0
<u>S.cubana</u>	0	95.0	91
	4	95.0	
	8	95.0	0
<u>S.gaminara</u>	0	96.0	96.5
	4	95.0	
	8	95.0	0
<u>S.urbana</u>	0	96.0	97
	4	96.0	
	8	96.0	0
<u>S.manhattan</u>	0	95.5	95
	4	95.0	
	8	95.0	0
<u>S.montevideo</u>	0	95.5	85.5
	4	95.0	
	8	95.0	0

(a) Per cent transmittance at a wave length of 620 millimicrons.

(b) Mean value of duplicate plate counts.

Table 15. EFFECT OF INCUBATION AT 131°F ON THE SURVIVAL OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	No. of survivors per ml. following exposure of ^(a) :		
	0 min.	15 min.	30 min.
<u>S.typhimurium</u> 6087	106,500,000	438	3
<u>S.senftenberg</u> 775W	115,500,000	Too many to estimate.	Too many to estimate.
<u>S.enteritidis</u>	124,750,000	>2,000	36
<u>S.cubana</u>	115,000,000	>2,000	>1,000
<u>S.gaminara</u>	105,500,000	334	9
<u>S.manhattan</u>	116,750,000	1	0
<u>S.montevideo</u>	101,500,000	1	0

(a) Mean value of duplicate plate counts.

Table 16. EFFECT OF INCUBATION AT 140°F ON THE SURVIVAL OF SALMONELLAE IN BRAIN HEART INFUSION BROTH

Organism	No. of survivors per ml. following exposure of ^(a) :		
	0 min.	15 min.	30 min.
<u>S. typhimurium</u> 6087	94,000,000	0	0
<u>S. senftenberg</u> 775W	105,000,000	75	0
<u>S. enteritidis</u>	107,500,000	0	0
<u>S. cubana</u>	120,500,000	0	0
<u>S. gaminara</u>	123,000,000	0	0
<u>S. manhattan</u>	107,500,000	0	0
<u>S. montevideo</u>	103,000,000	0	0

(a) Mean value of duplicate plate counts.

APPENDIX B

Growth of selected strains of salmonellae and staphylococci in refrigerated foods.

Table 1. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN GUSTARD INCUBATED AT VARIOUS TEMPERATURES^(a)

Incubation Temperature.	Sample No.	Number of organisms per gram $\times 10^{-6}$ after incubation for: (b)(c)									
		Salmonellae					Staphylococci				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
40°F	1	9.2 8.5	6.1 7.5	4.3 5.1	5.3 4.2	3.9 3.2	15.4 14.8	15.5 14.4	15.2 15.6	15.3 16.5	13.5 16.9
	2	8.7 7.6	5.5 6.2	3.0 4.8	2.4 2.1	3.8 3.4	14.8 12.7	12.7 14.1	14.9 13.2	13.4 12.9	16.4 19.4
42°F	1	8.5 5.4	6.0 5.3	3.7 4.4	4.4 3.9	3.3 3.4	13.1 9.2	15.5 13.9	12.6 10.7	15.4 15.0	11.6 11.6
	2	9.0 7.1	8.4 5.1	3.8 6.0	3.0 3.3	3.0 2.4	16.8 15.1	11.7 13.8	14.1 13.3	12.0 12.9	10.1 14.8
44°F	1	11.5 8.7	5.4 6.7	4.2 5.3	4.2 4.5	2.0 2.0	16.4 15.7	14.0 12.9	13.2 12.3	15.4 17.6	18.2 20.5
	2	9.1 7.8	4.9 4.4	4.1 5.4	3.6 4.5	4.4 5.7	14.1 15.7	14.5 14.2	13.1 15.8	16.1 16.7	18.6 17.2
46°F	1	9.3 9.9	7.3 9.1	4.3 5.8	5.0 4.6	2.2 2.6	13.7 13.5	12.7 15.4	13.8 13.8	27.2 26.2	52.0 47.0
	2	8.9 9.0	6.2 5.8	3.7 3.5	4.1 4.4	2.5 2.8	14.9 16.6	11.8 14.1	15.5 14.9	24.5 26.0	47.0 54.0
48°F	1	5.8 7.1	5.9 5.0	4.1 5.2	2.7 2.6	1.4 1.4	15.1 20.0	18.1 14.2	20.6 20.7	77.0 87.0	216.0 216.0
	2	5.6 5.3	4.8 3.9	3.6 4.5	2.6 2.9	1.2 1.3	15.4 16.2	16.2 16.8	21.2 20.2	75.0 75.0	293.0 270.0
50°F	1	11.0 10.0	6.2 8.3	5.3 7.2	2.2 2.5	2.0 2.1	13.2 13.5	18.0 19.9	75.0 68.0	400.0 450.0	1,360.0 1,380.0
	2	6.9 7.7	4.3 5.1	3.7 3.1	2.5 2.0	1.7 2.0	16.1 16.6	17.5 16.2	79.0 69.0	360.0 240.0	880.0 830.0
95°F	1	780.0 890.0	560.0 590.0	600.0 630.0	790.0 810.0	1,520.0 1,600.0	2,360.0 2,550.0	1,330.0 1,440.0	1,460.0 1,580.0	1,090.0 1,140.0	3,700.0 5,600.0
	2	750.0 710.0	630.0 520.0	610.0 660.0	690.0 780.0	2,500.0 1,250.0	1,870.0 1,240.0	1,360.0 1,560.0	1,510.0 1,470.0	1,570.0 1,590.0	2,220.0 2,010.0

(a) Inoculum consisted of a pooled suspension of approximately equal numbers of *Salmonella senftenberg* 775W, *S. enteritidis*, and *S. manhattan*, and a pooled suspension of approximately equal numbers of *Staphylococcus aureus* 19CE, *Staph. aureus* Ms 149, and *Staph. aureus* MF 159.

(b) Number of organisms recovered from food immediately after inoculation: salmonellae = $12.1-16.6 \times 10^6/\text{gm.}$; staphylococci = $12.0-16.3 \times 10^6/\text{gm.}$

(c) All uninoculated control jars of food were negative for growth.

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Table 2. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN CHICKEN A LA KING INCUBATED AT VARIOUS TEMPERATURES^(a)

Incuba- tion Temper- ature	Sample No.	Number of organisms per gram x 10 ⁻⁶ after incubation for: (b)(c)									
		Salmonellae					Staphylococci				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
40°F	1	13.5 12.9	11.6 11.7	12.8 12.8	12.7 11.5	15.9 15.8	15.0 17.4	15.8 18.1	15.4 15.8	14.2 14.5	15.5 16.2
	2	13.9 14.1	12.9 12.1	12.8 13.7	12.8 13.6	12.8 11.3	13.8 13.2	12.1 12.5	11.3 13.9	12.9 13.7	11.6 11.4
42°F	1	11.0 12.8	9.8 10.0	11.1 13.6	12.6 11.8	17.4 21.7	16.2 15.7	15.7 14.1	18.9 18.2	15.4 13.7	13.0 9.9
	2	12.7 14.1	11.5 13.6	9.6 12.4	12.9 14.2	10.1 10.4	11.9 9.4	12.9 10.3	15.3 14.4	14.0 14.8	13.9 13.2
44°F	1	15.7 15.3	13.6 13.1	15.0 13.2	18.5 19.8	61.0 48.0	15.2 16.6	16.6 18.2	17.3 15.7	19.7 20.3	24.7 22.4
	2	15.4 15.1	12.7 12.7	17.4 16.2	22.0 21.8	102.0 97.0	17.1 15.5	19.1 16.5	19.9 20.1	17.2 16.9	27.2 27.8
46°F	1	13.4 12.7	10.7 12.4	19.5 18.5	38.0 50.0	116.0 119.0	13.0 15.3	14.0 14.8	18.3 18.6	16.8 15.5	26.1 26.1
	2	18.4 16.4	16.0 16.4	19.7 20.9	49.0 60.0	124.0 132.0	16.8 16.8	18.3 13.6	19.7 17.4	17.2 16.1	28.7 30.1
48°F	1	11.8 12.8	17.5 20.4	82.0 62.0	233.0 215.0	700.0 800.0	14.4 11.2	20.3 17.4	25.3 22.5	28.3 27.9	106.0 116.0
	2	17.1 15.7	16.2 21.3	49.0 59.0	176.0 183.0	580.0 670.0	17.2 15.5	16.0 17.2	19.8 16.4	22.9 24.3	39.0 46.0
50°F	1	14.5 11.3	42.0 54.0	281.0 262.0	570.0 610.0	690.0 730.0	23.1 21.9	38.0 36.0	32.0 39.0	450.0 410.0	370.0 510.0
	2	12.3 14.0	42.0 54.0	300.0 284.0	620.0 630.0	640.0 570.0	17.1 15.7	46.0 55.0	79.0 72.0	390.0 430.0	470.0 430.0
95°F	1	1,040.0 1,110.0	620.0 580.0	61.0 56.0	7.9 8.4	0.9 0.8	1,150.0 1,050.0	550.0 690.0	430.0 370.0	490.0 550.0	126.0 122.0
	2	1,490.0 1,510.0	500.0 530.0	94.0 101.0	2.1 2.1	1.0 0.9	980.0 960.0	580.0 590.0	309.0 330.0	440.0 450.0	147.0 172.0

(a) Inoculum consisted of a pooled suspension of approximately equal numbers of *Salmonella senftenberg* 775W, *S. enteritidis*, and *S. manhattan* and a pooled suspension of approximately equal numbers of *Staphylococcus aureus* 196E, *Staph. aureus* Ms 149, and *Staph. aureus* MF 159.

(b) Number of organisms recovered from food immediately after inoculation: salmonellae = 11.6-16.0 x 10⁶/gm.; staphylococci = 12.3-17.4 x 10⁶/gm.

(c) All uninoculated control jars of food were negative for growth.

Table 3. GROWTH OF SALMONELLAE AND STAPHYLOCOCCI IN HAM SALAD INCUBATED AT VARIOUS TEMPERATURES^(a)

Incuba- tion Temper- ature.	Sample No.	Number of organisms per gram $\times 10^{-6}$ after incubation for: (b)(c)									
		Salmonellae					Staphylococci				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
40°F	1	14.8 14.8	10.7 6.9	7.8 7.2	6.3 5.6	7.9 6.5	16.8 16.2	12.0 10.7	11.6 11.8	10.5 10.7	14.0 13.7
	2	14.4 16.0	7.1 7.4	8.9 8.2	5.6 5.9	6.5 5.6	14.3 13.0	11.9 11.2	11.1 8.9	12.5 12.9	14.2 15.7
42°F	1	14.0 14.8	9.2 9.5	7.7 8.0	5.9 5.9	4.5 4.5	16.0 14.2	10.5 10.2	9.7 9.4	7.6 6.2	18.7 18.4
	2	18.3 17.2	5.0 5.9	8.6 8.5	7.0 6.0	4.5 4.0	17.9 15.8	8.6 8.0	9.8 10.5	9.5 10.2	17.4 15.4
44°F	1	11.3 11.8	5.0 5.8	6.9 7.0	4.8 5.8	3.6 5.1	17.8 17.0	9.2 8.9	7.8 8.2	7.5 7.7	16.2 14.9
	2	11.9 11.1	5.6 5.5	7.6 6.8	5.3 5.1	4.8 3.2	12.1 12.5	9.1 9.1	7.8 8.4	11.8 12.6	16.7 18.4
46°F	1	17.8 16.8	8.2 8.6	5.8 5.9	4.5 5.7	5.1 5.8	12.8 10.6	10.5 11.8	10.5 10.6	11.2 11.0	18.0 12.8
	2	12.9 13.7	8.0 7.8	6.8 6.4	4.4 3.7	5.2 4.6	16.2 14.7	9.2 9.6	9.8 8.3	14.6 13.3	15.0 13.0
48°F	1	21.2 22.1	7.5 7.6	3.8 3.9	3.1 3.0	2.4 1.5	19.5 19.0	9.2 10.5	9.4 9.6	13.2 14.3	17.8 17.6
	2	17.8 18.0	7.4 7.7	4.5 4.2	5.4 5.0	4.2 3.9	20.5 13.7	10.4 10.8	10.9 11.9	16.1 16.3	18.6 16.6
50°F	1	12.6 14.8	8.1 9.1	7.8 8.1	6.1 5.9	8.1 6.5	16.7 17.1	13.4 10.6	12.3 11.5	11.2 11.8	16.5 20.0
	2	15.9 15.2	6.2 8.1	5.8 5.4	5.4 5.3	4.2 3.9	15.3 12.6	12.3 11.8	9.6 9.3	12.7 13.5	16.0 20.6
95°F	1	36.0 41.0	159.0 165.0	151.0 140.0	23.0 97.0	117.0 103.0	76.0 77.0	700.0 660.0	530.0 510.0	490.0 490.0	199.0 209.0
	2	28.0 37.0	158.0 155.0	146.0 138.0	95.0 21.0	115.0 121.0	49.0 57.0	750.0 620.0	580.0 610.0	560.0 550.0	200.0 180.0

(a) Inoculum consisted of a pooled suspension of approximately equal numbers of *Salmonella* senftenberg 775W, *S. enteritidis*, and *S. manhattan* and a pooled suspension of approximately equal numbers of *Staphylococcus aureus* 196C, *Staph. aureus* No. 149, and *Staph. aureus* NF 159.

(b) Number of organisms recovered from food immediately after inoculation: salmonellae = $12.0-14.4 \times 10^6/\text{gm.}$; staphylococci = $14.4-15.3 \times 10^6/\text{gm.}$

(c) All uninoculated control jars of food were negative for growth.